

CLAIM AMENDMENT

The following listing of claims replaces all previous listings or versions thereof:

1. (Previously presented) A method of obtaining a bacterium comprising a nucleic acid sequence encoding a binding polypeptide having specific affinity for a target ligand comprising the steps of:
 - (a) providing a Gram negative bacterium comprising an inner and an outer membrane and a periplasm, said bacterium expressing a nucleic acid sequence encoding a candidate binding polypeptide, wherein the candidate binding polypeptide is exposed within the periplasm of said bacterium;
 - (b) contacting the bacterium with a labeled ligand under conditions wherein the labeled ligand contacts the binding polypeptide; and
 - (c) selecting said bacterium based on the presence of said labeled ligand bound to said candidate binding polypeptide.
2. (Original) The method of claim 1, further defined as a method of obtaining a nucleic acid sequence encoding a binding polypeptide having a specific affinity for a target ligand, the method further comprising the step of:
 - (d) cloning a nucleic acid sequence encoding said candidate binding polypeptide from said bacterium.
3. (Previously presented) The method of claim 1, wherein said nucleic acid sequence is further defined as operably linked to a leader sequence that directs the expression of said fusion polypeptide to the outer side of the inner membrane.
4. (Original) The method of claim 1, wherein said Gram negative bacterium is an *E. coli* bacterium.
5. (Original) The method of claim 1, wherein step (a) is further defined as comprising providing a population of Gram negative bacteria.

6. (Original) The method of claim 1, wherein the candidate binding polypeptide is anchored to the outer side of the inner membrane of said bacterium.
7. (Original) The method of claim 5, wherein said population of bacteria is further defined as collectively expressing a plurality of nucleic acid sequences encoding a plurality of candidate binding polypeptides.
8. (Original) The method of claim 2, wherein the bacterium is non-viable.
9. (Original) The method of claim 2, wherein the bacterium is viable.
10. (Original) The method of claim 2, wherein cloning comprises amplification of the nucleic acid sequence.
11. (Original) The method of claim 7, wherein said plurality of nucleic acid sequences are further defined as encoding a fusion polypeptide comprising a candidate binding polypeptide and a polypeptide anchored to the to the outer side of the inner membrane of the bacterium.
12. (Previously presented) The method of claim 5, wherein said population of bacteria is produced by a method comprising the steps of:
 - (a) preparing a plurality of nucleic acid sequences encoding a plurality of fusion polypeptides comprising a candidate binding polypeptide and an inner membrane anchor polypeptide; and
 - (b) transforming a population of Gram negative bacteria with said DNA inserts.
13. (Original) The method of claim 5, wherein said population of Gram negative bacteria is contacted with said labeled ligand.
14. (Original) The method of claim 5, wherein selecting in step (c) is further defined as comprising at least two rounds of selecting, wherein a sub-population of bacteria is selected based on the presence of said labeled ligand bound to said candidate binding polypeptide and

further wherein the sub-population is subjected to at least one additional selection based on the presence of said labeled ligand bound to said candidate binding polypeptide.

15. (Original) The method of claim 14, wherein from about two to six rounds of selecting are carried out.

16. (Original) The method of claim 14, wherein selecting is carried out by flow-cytometry or magnetic separation.

17. (Original) The method of claim 1, wherein said candidate binding polypeptide is further defined as an antibody or fragment thereof.

18. (Original) The method of claim 17, wherein said candidate binding polypeptide is further defined as a scAb, Fab or scFv.

19. (Original) The method of claim 1, wherein said candidate binding polypeptide is further defined as a binding protein of at least 40 amino acids other than an antibody.

20. (Original) The method of claim 1, wherein said candidate binding polypeptide is further defined as comprising less than 39 amino acids.

21. (Original) The method of claim 1, wherein said candidate binding polypeptide is further defined as an enzyme.

22. (Currently amended) The method of claim 1, wherein said labeled ligand is selected from the group consisting of a peptide, a polypeptide, an enzyme, a nucleic acid, a small molecule (< 1 kDa) and a synthetic molecule.

23. (Original) The method of claim 1, wherein said labeled ligand is further defined as fluorescently labeled.

24. (Previously presented) The method of claim 1, wherein said nucleic acid encoding a candidate binding polypeptide is further defined as flanked by known nucleic acid sequences.
25. (Original) The method of claim 1, further comprising treating said bacterium to increase the permeability of the outer membrane of said bacterium to said labeled ligand.
26. (Original) The method of claim 25, wherein treating comprises a method selected from the group consisting of: treatment with hyperosmotic conditions, treatment with physical stress, infecting the bacterium with a phage, treatment with lysozyme, treatment with EDTA, treatment with a digestive enzyme and treatment with a chemical that disrupts the outer membrane.
27. (Original) The method of claim 26, wherein treating comprises a combination of said methods.
28. (Original) The method of claim 27, wherein treating comprises treatment with lysozyme and EDTA.
29. (Original) The method of claim 25, wherein treating comprises treating the bacterium with a combination of physical, chemical and enzyme disruption of the outer membrane.
30. (Original) The method of claim 1, wherein said bacterium comprises a mutation conferring increased permeability of said outer membrane to said labeled ligand.
31. (Original) The method of claim 1, further comprising removing the outer membrane of said bacterium.
32. (Original) The method of claim 1, wherein said bacterium is grown at a sub-physiological temperature.
33. (Original) The method of claim 32, wherein said sub-physiological temperature is about 25°C

34. (Original) The method of claim 1, further comprising removing labeled ligand not bound to said candidate binding polypeptide.
35. (Original) The method of claim 1, further defined as comprising contacting the bacterium with at least two labeled ligands.
36. (Original) The method of claim 1, wherein said selecting comprises flow cytometry.
37. (Original) The method of claim 1, wherein said selecting comprises magnetic separation.
38. (Original) The method of claim 1, wherein said ligand and said candidate binding polypeptide are reversibly bound.
39. (Original) The method of claim 6, wherein the polypeptide anchored to the outer side of the inner membrane comprises a transmembrane protein or fragment thereof.
40. (Original) The method of claim 6, wherein the polypeptide anchored to the outer side of the inner membrane comprises a sequence selected from the group consisting of: the first two amino acids encoded by the *E. coli* NlpA gene, the first six amino acids encoded by the *E. coli* NlpA gene, the gene III protein of filamentous phage or a fragment thereof, an inner membrane lipoprotein or fragment thereof.
41. (Original) The method of claim 6, wherein the polypeptide anchored to the outer side of the inner membrane is anchored via an N- or C-terminus of the polypeptide.
42. (Original) The method of claim 40, wherein the sequence is an inner membrane lipoprotein or fragment thereof selected from the group consisting of: AraH, MglC, MalF, MalG, Mal C, MalD, RbsC, RbsC, ArtM, ArtQ, GlnP, ProW, HisM, HisQ, LivH, LivM, LivA, Liv E, Dpp B, DppC, OppB, AmiC, AmiD, BtuC, FhuB, FecC, FecD, FecR, FepD, NikB, NikC, CysT,

CysW, UgpA, UgpE, PstA, PstC, PotB, PotC, PotH, PotI, ModB, NosY, PhnM, LacY, SecY, TolC, DsbB, DsbD, TonB, TatC, CheY, TraB, ExbD, ExbB and Aas.

43. (Previously presented) A method of obtaining a bacteria comprising a nucleic acid sequence encoding at least a first binding polypeptide having specific affinity for a target ligand comprising the steps of:

- (a) providing a Gram negative bacterium comprising an inner and an outer membrane and a periplasm, said bacteria expressing a nucleic acid sequence encoding a least a candidate binding polypeptide, wherein the candidate binding polypeptide is exposed within the periplasm of said bacterium;
- (b) contacting the bacterium with a fluorescently labeled ligand under conditions wherein the labeled ligand contacts the binding polypeptide; and
- (c) selecting said bacterium for the presence of the fluorescently labeled ligand using Fluorescence Activated Cell Sorting (FACS).

44. (Original) The method of claim 43, further defined as a method of obtaining a nucleic acid sequence encoding a binding polypeptide having a specific affinity for a target ligand, the method further comprising the step of:

- (d) cloning a nucleic acid sequence encoding said candidate binding polypeptide from said bacterium

45. (Original) The method of claim 43, further defined as comprising providing a population of Gram negative bacteria.

46. (Original) The method of claim 45, wherein said population of bacteria is further defined as collectively expressing a plurality of nucleic acid sequences encoding a plurality of candidate binding polypeptides.

47. (Original) The method of claim 43, wherein the candidate binding polypeptide is anchored to the outer side of the inner membrane of said bacterium.

48. (Previously presented) A method of obtaining a nucleic acid sequence encoding a binding polypeptide having a specific affinity for a target ligand comprising the steps of:

- (a) providing a Gram negative bacterium comprising an inner and an outer membrane and a periplasm, said bacterium expressing a nucleic acid sequence encoding a candidate binding polypeptide, wherein the candidate binding polypeptide is anchored to the outer side of the inner membrane of said bacterium;
- (b) disrupting the outer membrane of said bacterium;
- (c) contacting the bacterium with a labeled ligand under conditions wherein the labeled ligand contacts the binding polypeptide;
- (d) selecting said bacterium based on the presence of said labeled ligand bound to said candidate binding polypeptide; and
- (e) cloning a nucleic acid sequence encoding said candidate binding polypeptide from said bacterium.